SUPPLEMENTARY TEXT 10

ASSOCIATION OF CHANGES (BETWEEN THE FINAL AND BASELINE TIME-POINTS) IN MICROBIOME INDEX WITH DIFFERENT MEASURES.

To further illustrate the links between diet, microbiome, and health, we investigated the associations of the across time-point changes of the various measures with the change in diet and microbiome. The change in microbiome index was not associated with either baseline dietary adherence scores (linear regression R=-0.034; P < 0.31) or the 12-month dietary adherence scores (linear regression R = 0.05; P < 0.12) (**Supplementary figure 15a, b**). We first performed an in-depth investigation of the association of the across-time-point (follow-up to baseline) changes in cytokine levels for each individual with the corresponding change in microbiome indices. Cumulated levels of anti-inflammatory cytokines were calculated as the summed ranked abundances of the anti-inflammatory cytokines (IL-10, IL-4, IL-5 and IL-1ra). Ratio of hsCRP levels to anti-inflammatory cytokine levels was calculated as the ratio of the ranked abundance of hsCRP to the cumulated levels of anti-inflammatory cytokines (calculated as above). Then for each cytokine (or cytokine ratio), the changes were calculated as the differences in levels between the follow-up and the baseline time-points. For inflammatory markers like hsCRP, MCP1-MCAF, Resistin, positive changes in microbiome indices were associated with significant negative changes in the levels of these cytokines (**Supplementary figure 15c**). For other inflammatory cytokines like IL-17, IL-6, MIP-1b, etc, the associations were still negative, although not significant. An exact opposite trend was observed for the anti-inflammatory cytokine IL-10, where positive changes in microbiome indices were associated with significant positive changes in the levels of this cytokine. As a consequence, positive changes in microbiome indices were associated with negative changes in the hsCRP to anti-inflammatory cytokine levels (**Supplementary figure 15c**).

Additionally, a pairwise regression approach was also used to identify associations between microbiome response, adherence score changes and the identified measures of frailty, cognitive function and inflammation. Given any two measures, we performed linear regressions of the measures with each of the scores using country and age as confounders. We did not use FDR correction at this stage as we were investigating associations with specific measures. Linear relationships with P-values less than 0.05 and between 0.1 and 0.05 were identified as being significant and trend, respectively.

The significant associations identified from this analysis are illustrated in **Supplementary figure 15d)** As expected, microbiome response was positively associated with dietary adherence changes. However, it was this increased microbiome response that displayed positive associations with reduced frailty (Reduced Fried Score), improved cognitive function (BabCock Memory Score) and negative associations with inflammation (hsCRP and another pro-inflammatory marker MIP-1b). The adherence score change, by itself, did not have any significant association (with exception of a negative association with MIP-1b). The above results clarify the relationships between diet, microbiome and improved life-status. Change in adherence (that is increasing adherence to a Mediterranean diet) is likely to modulate specific components of the microbiome. It is this microbiome response, when induced, that is associated with reduced frailty and reduced inflammation. However, at each interaction point, there may be exceptions.